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Title: Melatonin improves sleep in children with epilepsy: randomized, double-blind cross-over study

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Abbreviations:
Wakefulness after sleep onset (WASO)
Mean difference (MD)
Sustained release (SR)
Antiepileptic drugs (AEDs)
Sleep behavior questionnaire (SBQ)
Periodic limb movement (PLM)
Electroencephalogram (EEG)
Sleep onset latency (SL)
American Academy of Sleep Medicine (AASM)
Sleep efficiency (SE),

Psychomotor vigilance task (PVT).
Reaction times (RT),
Children-Parent Rating Scales (BASC-PRS)
Quality of Life in Childhood Epilepsy (QOLCE)
Standard deviation [SD]
Apnea hypopnea index (AHI), obstructive index (OI)
Periodic limb movement index (PLMI)

Highlights

We performed a Class I study using melatonin as a hypnotic in children with epilepsy.

In the study, melatonin improved sleep latency and wakefulness after sleep onset.

No significant increase was seen in seizure frequency or spike density; however, the study was too small to allow any conclusions to be drawn in this regard.

Increased N3 and reduced REM sleep were noted on melatonin compared to placebo.

Abstract

Objective: Insomnia, especially maintenance insomnia is widely prevalent in epilepsy. Although melatonin is commonly used, limited data address its efficacy. We performed a randomized, double-blind, placebo-controlled, cross-over study to identify the effects of melatonin on sleep and seizure control in children with epilepsy.

Methods: Eleven pre-pubertal, developmentally normal children aged 6-11 years with epilepsy were randomized by software algorithm to receive placebo or 9 mg sustained release melatonin for 4 weeks, followed by a 1-week washout and 4-week crossover condition. The pharmacy performed blinding; patients, parents and study staff other than a statistician were blinded. Primary outcomes

were sleep onset latency and wakefulness after sleep onset (WASO) measured on polysomnography. Secondary outcomes included seizure frequency, epileptiform spike density per hour of sleep on EEG and reaction time measures on psychomotor vigilance task. Statistical tests appropriate for cross-over designs were used for analysis.

Results: Data were analyzed from ten subjects who completed the study. Melatonin decreased sleep latency (Mean difference (MD): 11.4 min, p= 0.02) and WASO (MD 22 min, p=0.04) as compared to placebo. No worsening of spike density or seizure frequency was seen. Additionally, Slow-wave sleep duration and REM latency were increased with melatonin and REM sleep duration was decreased. These changes were statistically significant. Worsening of headache was noted in one subject with migraine on melatonin.

Conclusion: Sustained-release melatonin resulted in statistically significant decreases in sleep latency and WASO. No clear effects on seizures were observed but the study was too small to allow any conclusions to be drawn in this regard.

Key words: 1. Seizure frequency, 2. Sleep latency, 3. WASO, 4. Sleep architecture 5. Natural supplement 6. EEG spikes

1 Introduction:

Epilepsy affects 1% of the population in the United States.[1] Sleep related comorbidities are considerably higher in children with epilepsy than in unrelated healthy controls[2] and in patients with nocturnal seizures and refractory epilepsy.[3] In adults with epilepsy, 55% have insomnia[4]; 34% have sleep onset insomnia while 52% have maintenance insomnia.[5]

Melatonin is considered in treatment of circadian rhythm disorders, jet leg disorders and shift work sleep disorders. However, it has been widely used as a hypnotic in children with neurological disorders, including patients with epilepsy.[6] There is evidence that patients with epilepsy, especially refractory epilepsy, have reduced melatonin levels.[7-9] However, it is not clear whether exogenous melatonin improves sleep in children with epilepsy, or affects seizure control or daytime functioning.

We performed this study to fill this literature gap, using a sustained release (SR) melatonin formulation because of concerns about not only sleep onset, but also sleep maintenance insomnia in this population.

2 Methods:

Our primary research question was "does melatonin shorten sleep onset latency and reduce wakefulness after sleep onset (WASO) in children with epilepsy as compared to placebo".

This study was approved by the institutional review board (IRB) at the Cincinnati Children Hospital Medical Center (CCHMC). Written informed consent was obtained for all subjects from parents or legal guardians and assent from subjects 11 years old. The study was registered with ClinicalTrials.gov (NCT00965575).

2.1 Trial design

This was a randomized, double-blind, placebo-controlled, cross-over study using sustained release (SR) melatonin at 9 mg dose. There is limited data for use of melatonin in children with epilepsy. In pediatric clinical practice, doses as high as 18 mg have been used.[10] We selected 9 mg dose, as doses of 9-10 mg have been used safely, effectively and well tolerated in children with epilepsy in other studies.[11-13] As this study assessed the hypnotic effects of melatonin, the dose was given 30 min prior to bedtime. Antiepileptic drugs

(AEDs) were maintained at stable doses throughout the study. Adverse events were determined at each in-person/phone call visit and recorded in the subject's chart. Our study was originally designed to allow enrollment of subjects with refractory epilepsy, but this proved difficult due to inability to keep their AEDs at stable dosages, as well as the common presence of cognitive, psychiatric or developmental comorbidities, which were enrollment exclusions. Hence, it was difficult to find and recruit subjects for this reason. Also, due to three overnight sleep studies, most parents preferred the study being completed during summer vacations, which also caused delay in recruitment.

Study participant disposition is summarized in Figure 1. The study design is shown in the Figure 2. After baseline testing and eligibility determination, subjects were randomized to receive placebo or melatonin for four weeks. After 1 week of washout, the subjects who were initially placed on placebo received melatonin for the next four weeks, and vice versa. Outcomes were collected at baseline and at the end of each of the treatment phases.

2.3 Participants

Subjects were enrolled from the clinics at the Cincinnati Children's Hospital Medical Center.

Inclusion Criteria: Six to eleven years old pre-pubertal (Tanner stage I) children with epilepsy, with normal development based on school placement (in appropriate grade based on age) and developmental history or IQ>70, were screened with sleep behavior questionnaire (SBQ).[2] A combined score of 30, or more on sleep fragmentation, parasomnia and daytime drowsiness subscales was required for enrollment. We enrolled pre-pubertal children to avoid patients with potential delayed sleep phase syndrome where melatonin may have a phase advancing effect.

Exclusion Criteria: Subjects were excluded if they had a history of loud snoring, diagnosis of obstructive sleep apnea [obstructive apnea hypopnea index >2/hour] or periodic limb movement (PLM) disorder [PLM Index >5/ hour] on polysomnography. We also excluded patients with Vagus nerve stimulator, history of a major psychiatric disease, pervasive development disorder, severe neuro-developmental disabilities, immune disorders or lympho-proliferative disorders. Concurrent use of hypnotics, stimulants, systemic corticosteroids or other immuno-suppressants, or history of using SR melatonin was also exclusionary.

2.4 Intervention

Sustained release melatonin formulation (Jigsaw Health, Scottsdale, AZ, [IND # 106,139]) at the dose of 9 mg was used as the active study medication which has a half-life of 6 hours as suggested by the manufacturer. As melatonin is predominantly metabolized in the liver by cytochrome P450 CYP1A2 and less importantly by CYP2C19 and CYP2C9, there may be individual differences in the metabolism.[14; 15]

If a subject experienced a severe study medication related adverse event, the dose could be reduced to 6 mg. The study medication was to be given about 30 minutes before bedtime.

2.5 Outcomes

All outcomes were performed at the baseline and at the end of each treatment phase.

The two pre-specified primary outcomes were sleep onset latency (SL) and wakefulness after sleep onset (WASO). SL was defined as time in minutes from lights out to sleep onset. WASO was defined as the sum of wake time minutes from sleep onset to the final awakening. Polysomnography was performed with Grass-Telefactor system. Subjects were studied for up to 12 hours in a quiet dark

room with an ambient temperature of 24 degree C, in the company of their parents. Subjects went to bed at their routine bedtime. The following parameters were recorded simultaneously: body position, bilateral electro-occulogram, six channels electroencephalogram, chin and anterior tibialis electromyogram, tracheal microphone, electrocardiogram pulse oximetry (Maximo), thoracic and abdominal inductance plethysmography, nasal pressure transducer and end-tidal CO2. Sleep stages were scored by a certified sleep technologist and SVJ who were blinded to the subject groups and according to the American Academy of Sleep Medicine (AASM) guidelines.[16] AASM Standard definitions were used for sleep efficiency (SE), total sleep time, percentages of sleep stages (N1, N2, N3, or REM %), arousals and arousal Index (AI). We performed single-night polysomnography as studies have shown no significant difference between first and second night in SE, SL, and WASO in patients with epilepsy.[17; 18]

The pre-specified secondary outcomes were seizure frequency as measured by seizure diary, epileptiform spike density per hour of sleep on EEG, reaction time measures as measured by psychomotor vigilance task (PVT). The other measures were total sleep time on actigraphy, subjective sleep as measured by sleep behavior questionnaire (SBQ) and sleep diary, salivary melatonin levels, behavior and quality of life as measured by questionnaires.

Seizure frequency was determined by parent/caregiver report on a seizure diary which was filled out after each seizure which is an accepted patient-reported outcome measure.[19]

EEGs utilized the international 10–20 placement of electrodes to collect overnight video EEGs. Epileptiform spikes were defined as sharply countered waveform with duration of 20 to 200 ms on EEG. Due to technical problems, the overnight EEGs were not available in entirety for some subjects. Hence, the spikes were counted from the 6 channel EEGs from the polysomnography manually by SVJ who is a pediatric epileptologist with significant clinical experience. This approach has been successfully used in another study.[20] The morphology and localization was confirmed with the 20-channel EEGs. Multiple discharges in one second or a burst of discharges lasting 1 second was counted as one. Synchronous discharges occurring in different regions in 1 second were counted as one. Independent multifocal discharges occurring in 1 second were counted separately. Spikes occurring during the awake epochs were not calculated. The spike density is reported as spikes per hour of sleep.

Reaction times were determined using the Psychomotor Vigilance Task (PVT). The PVT is a valuable test to study the impact of sleep deprivation and sleep disorders in adults and children.[21; 22] This test is a 10 minute long sustained attention task using visual stimulus. Poor performance is characterized by slower (higher) mean reaction times (RT), higher RT associated with the slowest ten percentages of responses and greater variability in RT. The PVT-192 Monitor from Ambulatory Monitoring, Inc. was used to assess these parameters.

Sleep Behavior Questionnaire (SBQ) includes a set of six questions related to sleep-wake habits and a 29-item Likert-type rating scale. The subscales include parasomnias, parent/child interaction, sleep fragmentation, daytime drowsiness and bedtime difficulties. The SBQ total score is considered a global index of sleep problems, with higher scores representing more sleep problems.[2; 23] Scores were

used for enrollment and as a subjective sleep measure. We chose this questionnaire as it provided detailed measure of insomnia related complaints.

Parents were also asked to complete a sleep diary recording bedtime, wake up times and nap times and duration.

The actigraph is a wristwatch-like device that uses a piezo-electric beam to detect movements based on which sleep wake cycles are determined.[24] Basic Motionlogger from Ambulatory Monitoring, Inc. was used to derive secondary measures of total sleep time, SL, sleep duration and WASO during 2 weeks before randomization and during the last week of treatment phases.

Salivary melatonin levels were obtained three times at each study visit- at the time of admission, 30 min before bedtime and in the morning after awakening.[25]

Behavior Assessment System for Children-Parent Rating Scales (BASC-PRS) and Quality of Life in Childhood Epilepsy (QOLCE) were filled by the parents to assess behavior and quality of life at each visit.[26; 27] Subscale scores were used to compare difference between melatonin and placebo.

2.6 Sample size

While sleep efficiency was a secondary outcome it was the only measure for which we had preliminary data. Thus, we based our power analysis on sleep efficiency assuming that the distribution of the changes in sleep latency would be similar. A sample size of

ten was calculated to confer a power of 0.92, using an alpha = 0.05, to detect a difference of 6.25% in sleep efficiency between melatonin and placebo (standard deviation [SD] of 5.15)[28] using a paired t-test. The sleep efficiency and SD data for sample size calculations were chosen from a study evaluating sleep in children with epilepsy, [28] while the effect size for the difference to be measured was chosen based on 6.25% increase in sleep efficiency in adults on eszopiclone.[29] Melatonin has been used in trials involving children with intellectual disabilities with and without epilepsy. However, these children have more severe sleep related problems. Hence, we did not think that the data from these studies were appropriate for current power analyses. This was a pilot study which was not powered to detect significant changes in secondary outcomes.

2.7 Randomization, concealment mechanism, implementation, and blinding

The Investigational Pharmacy at CCHMC performed the randomization by random number generators in www.randomization.com, ensured blinding via over- encapsulation of both the melatonin and placebo pills to have the same appearance, and dispensed the study medications. The pharmacy and the statistician were unblinded while the rest of the study team was blinded to the allocation throughout data collection, entry and cleaning.

2.8 Statistical methods

All outcome measures and analyses were pre-specified prior to breaking the blind. As a result of the cross-over design there are two groups consisting of those who received melatonin first and those who received placebo first. We first tested for the differences between the carry-over effects to rule out the effect of differential order of intervention. For those outcomes where differential cross-over effects were seen, results were then analyzed as differences from baseline for treatment phase 1 only. For all other outcomes, the carry-over effects were not significantly different. In these cases two-sample tests were conducted appropriate for

cross-over designs. In addition, and as a result of the cross-over design, the Wilcoxon Rank-Sum test was used, as opposed to the usual signed-rank test for paired-data.[30] = Since this was a pilot study and some secondary outcomes were collected to guide a future larger study, adjustments for multiple comparisons were not done for secondary outcomes.

3 Results:

The recruitment lasted from June 2011 to Dec 2012. Figure 1 represents CONSORT flow diagram for study screening, enrollment and analysis.

Thirteen subjects were enrolled, eleven were randomized, ten (91%) completed the entire study and served the cohort for analysis. The individual study subject characteristics including type of epilepsy, AEDs and MRI results are listed in the Table 1.The analysis cohort characteristics and baseline data are listed in the Table 2. All subjects tolerated SR melatonin at 9 mg dose. Adherence was assessed at more than 93% by counting tablets remaining at each treatment visit. Data was available for all ten subjects for the primary and secondary outcomes except for the PVT and sleep diary data (n=9) and actigraphy (n=8) due to technical problems. Average baseline apnea hypopnea index (AHI), obstructive index (OI) and periodic limb movement index (PLMI) were 0.8 ± 0.7, 0.6± 0.7, 0.6 ± 1.1 respectively.

3. 1Primary Outcomes: SR melatonin reduced mean sleep latency by 11 min (p=0.02,) and mean WASO by 22 min (p=0.04,) as compared to placebo (Table 3).

3.2 Secondary Outcomes and sleep architecture and subjective sleep measures: (all times quoted in this section are mean values for the 10 subjects) Based on sleep diary, subjects slept 11 minutes more (p=0.1) and woke up 18 minutes later (p=0.048) as compared to placebo. Bedtime was not significantly different between placebo and melatonin. Melatonin increased N3% by 5.5% (p= 0.04) and REM latency by 58 min (0.04) and reduced REM% by 5.8% (p=0.01) as compared to placebo (Table 3). Melatonin was associated with increase in sleep efficiency by 3.8% and total sleep time on actigraphy by 23 minutes, and decrease in spike density by 50/hour and mean seizure frequency by 1 seizure/month, which were non-significant differences from placebo. Additionally, no significant differences were seen for reaction times, actigraphy, behavior, quality of life or sleep questionnaires.

Serial salivary melatonin levels (Figure 3, Table 4) demonstrated similar melatonin trajectories between the baseline and placebo treatments. Higher melatonin levels were demonstrated while on the melatonin treatment.

3.4 Ancillary analyses

No subgroup analysis was done due to heterogeneity of the sample.

3.5 Harms

Four subjects reported adverse events while taking melatonin as compared to two subjects on placebo. One subject on melatonin with a history of migraine had increased severity of headache. Unrelated adverse events on melatonin were bronchitis and ear infection (one subject), agitation (one subject) and increased urinary frequency (one subject, continued from placebo phase) and on placebo were agitation (one subject, continued from melatonin phase) and increased urinary frequency (one subject).

4 Discussion:

This study showed that sustained release melatonin improves sleep latency (p= 0.02), and wakefulness after sleep onset (WASO) (p=0.04) in developmentally normal, 6-11 years old, pre-pubertal children with epilepsy, as compared to placebo at 4 weeks of treatment. Additionally, melatonin improves N3 but prolongs REM latency and reduces REM sleep. Melatonin did not appear to worsen seizure frequency or spike density; both epilepsy severity indexes were slightly (non-significantly) better during melatonin treatment than placebo.

We used sleep behavior questionnaire score for initial screening for enrollment.[2] We excluded the subscale score "parent/child interaction during night" from the enrollment criteria, as we wanted to exclude the subjects with behavioral insomnia. The enrolled subjects had difficulty initiating and/or maintaining sleep. Although, the parasomnia subscale was included in the initial enrollment, the subjects did not have a diagnosis of parasomnia. We also ruled out other sleep disorders such as sleep apnea or periodic limb movement disorder prior to randomization with a clinical evaluation and polysomnography. Hence, the enrolled subjects had insomnia. Additionally, we selected sleep latency and WASO as primary outcomes as they represent independent and equally important outcomes. These two outcomes are clinically relevant for treatment of onset and maintenance insomnia with a hypnotic. Since sleep efficiency is dependent on these variables, it was selected as a secondary outcome. Seizure frequency and spike density were also selected as secondary outcomes due to lack of preliminary data. Moreover, as the prevalence of maintenance insomnia is higher in patients with epilepsy[5] and SR melatonin has been shown to increase total sleep time[31], we decided to evaluate SR melatonin

formulation. Furthermore, melatonin doses up to 9-10 mg have been used in studies safely and effectively[11-13] and up to 18 mg have been used in clinical practice[10] Hence, we selected 9 mg dose.

4. 1 Melatonin and sleep architecture

Studies evaluating melatonin as a hypnotic, in children with intellectual disability and autism spectrum disorder, concluded that it reduced sleep latency and increased total sleep times.[31; 32 33] Only a few randomized studies specifically enrolled subjects with epilepsy. In a study in children, significant differences were seen on sleep questionnaire in median percent reduction in total sleep score and parasomnia score between melatonin and placebo.[13] In another study in nine to 32 year-old subjects, no improvement was seen on actigraphy, or sleep diary.[12] In the third study, sleep latency was reduced on melatonin on sleep logs.[11] Moreover, in much larger studies in adults with insomnia, on ramelteon sleep latency improved by 9.5 minutes, [34] on eszopiclone (at 3 mg dose) sleep latency improved by 22.5 and WASO by 13.7 minutes, [29] and on zolpidem sleep latency improved by 10 and WASO by 10 minutes as compared to placebo. [35] In our study, mean decrease in sleep latency was 11 minutes and WASO was 22 minutes, which is comparable to the above listed hypnotic studies. Even though we did not see a subjective improvement on sleep questionnaire, these seemingly small changes in sleep parameters were associated with subjective sleep improvement in these studies.[29; 34; 35] Additionally, there was some improvement in sleep efficiency (3.8%) on polysomnography, total sleep time on actigraphy, and sleep duration and later wake times based on sleep diary on melatonin. Moreover, N3% and REM latency increased while REM% was reduced significantly on melatonin. Several studies in healthy adults have reported increased REM sleep, reduced SWS and increased N2 with melatonin.[36] Prolonged REM latency has also been reported.[37] We believe that consolidated sleep due to reduction in WASO led to

improved N3%. This, along with prolonged latency to REM sleep, may have caused reduction in REM%. To our knowledge, this is the first study to evaluate the effect of sustained release melatonin in a randomized, double-blind, cross-over design, with objective outcomes, in children with normal development and epilepsy.

4.2 Melatonin and epilepsy

There is conflicting or unclear evidence about the effect of melatonin on epilepsy.[38-41] In a study of six children with multiple neurological disabilities, melatonin increased seizure frequency. In one subject upon stopping it, seizures improved while adding it again worsened seizures.[42] However, a recent randomized placebo-controlled cross-over study showed that melatonin improved diurnal seizure frequency in patients with refractory epilepsy.[12] Other studies report no worsening or occasional improvement.[38; 43] In our study, only two subjects had ongoing seizures; both had 50% reduction in seizure frequency on melatonin. Eight subjects who were seizure-free remained seizure-free during the study. Hence, we observed no worsening in seizure frequency. Due to inability to enroll children with refractory epilepsy, we were unable to evaluate the effect of melatonin on seizure frequency in detail. Moreover, in a previous study some improvement in EEG has been reported [43] but no other study has evaluated the EEG changes based on spike density. In our study, the spike density reduced by 50/hour of sleep on melatonin. Hence, we observed no worsening in EEG spikes on melatonin. Since our sample consisted of different types of epilepsy, there was significant variability in the spike densities (baseline mean ±SD-150.5 ± 234.8). This may be one of the factors for non-significant results, others being 80% of the subjects seizure-free and inadequate power for a secondary outcome.

4.3 Suggested mechanisms of melatonin

Melatonin is secreted by the pineal gland. There is increase in the melatonin level prior to sleep onset, and a peak is seen 4-5 hours after sleep onset. A reduction in intrinsic melatonin level has been reported in patients with epilepsy, [7-9] which may be the reason why melatonin supplementation improves sleep in these patients. Additionally, melatonin has been suggested to have GABA agonist effects [44] which may inhibit the arousal systems during sleep. Hypnotic agents, like Zolpidem, eszopiclone, zaleplon, are also GABA agonists. Other suggested mechanisms are the effects on supra-chiasmatic nucleus through MT1 receptors and thermoregulatory mechanism.[45; 46] Our results from salivary melatonin levels showed that the subjects were adherent with the active study drug. The plots were very similar for baseline and placebo phases and at higher levels during melatonin phase with similar curves as under physiological conditions.

We only performed one night polysomnography. Our study showed increased N3 and improved WASO, so we believe that there was not a significant sleep fragmentation due to first night effect. Prolonged REM latency has been reported with melatonin in a previous study.[37] Also, since 60% of the subjects in our study received melatonin first, the results of the study could be due to repeated polysomnography. Additionally, studies have shown no significant difference between first and second night in SE, SL, and WASO in patients with epilepsy.[17; 18]

4.4 Limitations

This was a small study, so the results may not be generalizable. As mentioned, we were unable to enroll subjects with refractory seizures due to which we were unable to detect significant differences in the epilepsy related outcomes. We did not see significant changes in actigraphy, reaction time, subjective sleep on questionnaire, or behavioral functioning. Total sleep time on actigraphy showed a trend towards improvement on melatonin. We lost some data for secondary outcomes from two subjects, which may have impacted the results. In our study, behavioral functioning was normal at the baseline visit, which may have limited the potential for change. Also, our study was not powered to detect moderate changes in these outcomes. We propose these can be the major reasons for non-significant differences in some secondary outcomes in our study. We did identify significant differences in key sleep parameters (primary outcomes) in an objective manner in a previously untested population.

4.5 Conclusions

Based on this study, we suggest that a larger scale study could address the efficacy for secondary outcomes, safety and tolerability in children with epilepsy.

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Neuropsychology Service through the Division of Behavioral Medicine and Clinical Psychology at Cincinnati Children's, providing and overseeing provision of clinical services to patients with neurological conditions, including epilepsy.

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References:

1. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N Engl J Med* 2000;342:314-319.

2. Cortesi F, Giannotti F, Ottaviano S. Sleep problems and daytime behavior in childhood idiopathic epilepsy. *Epilepsia* 1999;40:1557-1565.

3. Batista BH, Nunes ML. Evaluation of sleep habits in children with epilepsy. *Epilepsy Behav* 2007;11:60-64.

4. Vendrame M, Yang B, Jackson S, et al. Insomnia and epilepsy: a questionnaire-based study. *J Clin Sleep Med* 2013;9:141-146.

5. Khatami R, Zutter D, Siegel A, et al. Sleep-wake habits and disorders in a series of 100 adult epilepsy patients--a prospective study. *Seizure* 2006;15:299-306.

6. Jain SV, Simakajornboon N, Glauser TA. Provider practices impact adequate diagnosis of sleep disorders in children with epilepsy. *J Child Neurol* 2013;28:589-595.

7. Bazil CW, Short D, Crispin D, et al. Patients with intractable epilepsy have low melatonin, which increases following seizures. *Neurology* 2000;55:1746-1748.

8. Paprocka J, Dec R, Jamroz E, et al. Melatonin and childhood refractory epilepsy--a pilot study. *Med Sci Monit* 2010;16:CR389-396.

9. Yalyn O, Arman F, Erdogan F, et al. A comparison of the circadian rhythms and the levels of melatonin in patients with diurnal and nocturnal complex partial seizures. *Epilepsy Behav* 2006;8:542-546.

10. Heussler H, Chan P, Price AM, et al. Pharmacological and non-pharmacological management of sleep disturbance in children: an Australian Paediatric Research Network survey. *Sleep Med* 2013;14:189-194.

11. Coppola G, Iervolino G, Mastrosimone M, et al. Melatonin in wake-sleep disorders in children, adolescents and young adults with mental retardation with or without epilepsy: a double-blind, cross-over, placebo-controlled trial. *Brain Dev* 2004;26:373-376.

12. Goldberg-Stern H, Oren H, Peled N, et al. Effect of melatonin on seizure frequency in intractable epilepsy: a pilot study. *J Child Neurol* 2012;27:1524-1528.

13. Gupta M, Aneja S, Kohli K. Add-on melatonin improves sleep behavior in children with epilepsy: randomized, double-blind, placebo-controlled trial. *J Child Neurol* 2005;20:112-115.

14. Ma X, Idle JR, Krausz KW, et al. Metabolism of melatonin by human cytochromes p450. *Drug Metab Dispos* 2005;33:489-494.

15. Facciola G, Hidestrand M, von Bahr C, et al. Cytochrome P450 isoforms involved in melatonin metabolism in human liver microsomes. *Eur J Clin Pharmacol* 2001;56:881-888.

16. Iber C, American Academy of Sleep Medicine. The AASM manual for the scoring of sleep and associated events : rules, terminology and technical specifications. American Academy of Sleep Medicine: Westchester, IL; 2007.

17. Marzec ML, Selwa LM, Malow BA. Analysis of the first night effect and sleep parameters in medically refractory epilepsy patients. *Sleep Med* 2005;6:277-280.

18. Selwa LM, Marzec ML, Chervin RD, et al. Sleep staging and respiratory events in refractory epilepsy patients: Is there a first night effect? *Epilepsia* 2008;49:2063-2068.

19. NINDS. Epilepsy Common data elements- seizure diary Available at: http://www.commondataelements.ninds.nih.gov/Epilepsy.aspx#tab=Data_Standards Accessed 4/9/14.

20. Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med* 2010;363:1801-1811.

21. Lim J, Dinges DF. Sleep deprivation and vigilant attention. *Ann N Y Acad Sci* 2008;1129:305-322.

22. Venker CC, Goodwin JL, Roe DJ, et al. Normative psychomotor vigilance task performance in children ages 6 to 11--the Tucson Children's Assessment of Sleep Apnea (TuCASA). *Sleep Breath* 2007;11:217-224.

23. Byars AW, Byars KC, Johnson CS, et al. The relationship between sleep problems and neuropsychological functioning in children with first recognized seizures. *Epilepsy Behav* 2008;13:607-613.

24. Sadeh A. The role and validity of actigraphy in sleep medicine: an update. *Sleep Med Rev* 2011;15:259-267.

25. Shirakawa S, Tsuchiya S, Tsutsumi Y, et al. Time course of saliva and serum melatonin levels after ingestion of melatonin. *Psychiatry Clin Neurosci* 1998;52:266-267.

26. Allison Bender H, Auciello D, Morrison CE, et al. Comparing the convergent validity and clinical utility of the Behavior Assessment System for Children-Parent Rating Scales and Child Behavior Checklist in children with epilepsy. *Epilepsy Behav* 2008;13:237-242.

27. Sabaz M, Lawson JA, Cairns DR, et al. Validation of the quality of life in childhood epilepsy questionnaire in American epilepsy patients. *Epilepsy Behav* 2003;4:680-691.

28. Maganti R, Sheth RD, Hermann BP, et al. Sleep architecture in children with idiopathic generalized epilepsy. *Epilepsia* 2005;46:104-109.

29. Erman MK, Zammit G, Rubens R, et al. A polysomnographic placebo-controlled evaluation of the efficacy and safety of eszopiclone relative to placebo and zolpidem in the treatment of primary insomnia. *J Clin Sleep Med* 2008;4:229-234.

30. Fleiss JL. The design and analysis of clinical experiments. Wiley: New York; 1999.

31. Wasdell MB, Jan JE, Bomben MM, et al. A randomized, placebo-controlled trial of controlled release melatonin treatment of delayed sleep phase syndrome and impaired sleep maintenance in children with neurodevelopmental disabilities. *J Pineal Res* 2008;44:57-64.

32. Braam W, Smits MG, Didden R, et al. Exogenous melatonin for sleep problems in individuals with intellectual disability: a meta-analysis. *Dev Med Child Neurol* 2009;51:340-349.

33. Guenole F, Godbout R, Nicolas A, et al. Melatonin for disordered sleep in individuals with autism spectrum disorders: systematic review and discussion. *Sleep Med Rev* 2011;15:379-387.

34. Mayer G, Wang-Weigand S, Roth-Schechter B, et al. Efficacy and safety of 6-month nightly ramelteon administration in adults with chronic primary insomnia. *Sleep* 2009;32:351-360.

35. Roth T, Roehrs T, Vogel G. Zolpidem in the treatment of transient insomnia: a double-blind, randomized comparison with placebo. *Sleep* 1995;18:246-251.

36. Zhdanova IV, Lynch HJ, Wurtman RJ. Melatonin: a sleep-promoting hormone. *Sleep* 1997;20:899-907.

37. James SP, Sack DA, Rosenthal NE, et al. Melatonin administration in insomnia. *Neuropsychopharmacology* 1990;3:19-23.

38. Brigo F, Del Felice A. Melatonin as add-on treatment for epilepsy. *Cochrane Database Syst Rev* 2012;6:CD006967.

39. Sandyk R, Tsagas N, Anninos PA. Melatonin as a proconvulsive hormone in humans. *Int J Neurosci* 1992;63:125-135.

40. Banach M, Gurdziel E, Jedrych M, et al. Melatonin in experimental seizures and epilepsy. *Pharmacol Rep* 2011;63:1-11.

41. Stewart LS, Leung LS. Hippocampal melatonin receptors modulate seizure threshold. *Epilepsia* 2005;46:473-480.

42. Sheldon SH. Pro-convulsant effects of oral melatonin in neurologically disabled children. *Lancet* 1998;351:1254.

43. Jain S, Besag FM. Does melatonin affect epileptic seizures? *Drug Saf* 2013;36:207-215.

44. Zhdanova IV. Melatonin as a hypnotic: pro. *Sleep Med Rev* 2005;9:51-65.

45. Sack RL, Hughes RJ, Edgar DM, et al. Sleep-promoting effects of melatonin: at what dose, in whom, under what conditions, and by what mechanisms? *Sleep* 1997;20:908-915.

46. Pandi-Perumal SR, Srinivasan V, Spence DW, et al. Role of the melatonin system in the control of sleep: therapeutic implications. *CNS Drugs* 2007;21:995-1018.

Figure 1: CONSORT diagram

Figure 2: Study design

V: study visits, Diaries- Sleep diary, Seizure diary; Qs: questionnaires- BASC-PRS and SBQ and QOLCE; PVT: psychomotor vigilance task; EEG: electroencephalogram; PSG: polysomnography, SML: salivary melatonin level, Rnd: randomization, Blind: blinding

Figure 3: Mean salivary melatonin levels with standard error bars

The time points for collection were 1: at the time of the admission (mean 1705), 2: 30 min before sleep onset (mean 2112) and 3: upon awakening in the morning (mean 0732)

Table 1: Individual subject characteristics

Subjects	Epilepsy type	Antiepileptic medication	MRI results
#1	Focal	Lamotrigine, Zonisamide	Mild bright T2 signal was seen in the peri-atrial white matter bilaterally
# 2	Focal (BECTS)	Oxcarbazepine	Normal
# 3	Focal	Carbamazepine	Normal
# 4	Generalized (CAE)	Ethosuximide	NA
# 5	Focal (BECTS)	Levetiracetam	Normal
# 6	Focal	Oxcarbazepine	Normal

	(BECTS)		
# 7	Generalized (CAE)	Ethosuximide	Normal
# 8	Focal	Oxcarbazepine	Normal
# 9	Undetermined		A very small lipoma was noted below the mammillary bodies
# 10	Focal	Carbamazepine	Non-specific gliosis was seen

BECTS: benign epilepsy with centro-temporal spikes; CAE: childhood absence epilepsy, NA: not available

Table 2: Subject characteristics and pre-treatment (baseline) data

Parameter	Mean ± SD
Age (years)	8.4± 1.3
Gender Male (n, %)	7 (70%)
Ethnicity: Caucasian (n, %)	9 (90%)
Epilepsy Type: Focal: Generalized: Undetermined (n)	7: 2: 1
Sleep onset latency (in minutes)	23.6 ± 19.1
Wakefulness after sleep onset (in minutes)	60.6 ± 24

Psychomotor vigilance task (PVT): Mean reaction time (RT) in ms)	553.6 ± 280.0
PVT: RT variability	0.3 ± 0.3
PVT: Slower 10% of RT (in ms)	1169.7± 881.2
Spike Density (per hour of sleep)	150.5 ± 234.8
Seizure Frequency per month	0.9 ± 1.9
Sleep architecture measurements	
Sleep efficiency (%)	82.9 ± 5.9
Total sleep time (in minutes)	454.7 ± 62.8
Arousal index per hour of sleep	12.9 ± 7.4
REM Latency (in minutes)	136.2 ± 43.6
Stage N1%	6.3 ± 2.9
Stage N2%	42. 6 ± 5.2
Stage N3%	28.9 ± 3.4
Stage REM%	22.2 ± 3.7
Subjective sleep measures	
Sleep Behavior Questionnaire total score	59.2 ± 10.5
Bedtime (sleep diary time)	2144 ± 0.75

Wake up time (sleep diary time)	709 ± 1.04
Sleep duration (hour)	9.44 ± 0.50

SD: standard deviation, ms: milliseconds

Table 3: Primary and secondary outcome results from each phase of trial

	Placebo		Melatonin		Difference		p-value
Parameter	Mean	SD	Mean	SD	Mean	SD	
Primary outcomes							
Sleep onset latency (in minutes)	19.0	20.1	7.8	5.9	11.4	9.4	**0.02**
Wakefulness after sleep onset (in minutes)	57.3	31.6	42.3	30.3	22.2	16.7	**0.04**
Secondary outcomes							
Mean reaction time (RT) (in ms)	597.6	218.3	644.2	258.5	-41.8	76.6	0.90
RT variability	0.3	0.2	0.3	0.2	0.05	0.15	0.90
Slower 10% of RT (in ms)	1285.3	619.0	1812.7	1216.6	-545.5	595.5	0.54
Spike Density (per hour of sleep)	71.9	129.0	31.7	53.5	49.8	59.5	0.13
Seizure Frequency per month	2.4	6.6	1.4	3.8	1.2	1.4	0.53
Sleep architecture measures							
Sleep efficiency	86.8	5.8	89.2	6.1	-3.8	3.6	0.17

Total sleep time (in minutes)	496.4	40.2	458.3	58.5	39.3	31.7	0.26
Arousal index per hour of sleep	12.3	6.8	10.3	4.1	2.5	2.1	0.26
REM Latency (in minutes)	98.1	52.8	135.2	53.1	-58.3	30.9	**0.04**
Stage N1%[+]	4.8	2.8	4.0	2.0	-0.1	0.8	0.52
Stage N2%[+]	42.6	8.1	44.0	9.2	-2.9	4.0	0.39
Stage N3%	27.4	4.8	32.6	8.3	-5.5	3.0	**0.04**
Stage REM%	25.2	5.1	19.4	4.2	5.8	2.2	**0.01**
Total sleep time (in min)- actigraphy	511.1	33.5	532.4	38.4	-23.2	18.5	0.27
Subjective sleep measures							
Sleep Behavior Questionnaire*	48.3	7.4	52.4	5.4	-3.4	2.8	0.17
Bedtime (sleep diary time)	2149	0.72	2157	0.91	-6.8[#]	0.18	0.42
Wake up time (sleep diary time)	711	1.09	731	1.09	-18.1[#]	0.15	**0.048**
Sleep duration (hour)	9.34	0.60	9.54	0.48	-11.3[#]	0.15	0.10

SD: standard deviation, ms: milliseconds, [+] Significant carry over effects were seen, analysis performed as difference from baseline for phase 1 only, *Total scores, [#] in minutes

Table 4: Salivary melatonin levels (pg/ ml)

Time point (mean in military time)	Baseline Mean (Mean-SEM, Mean+ SEM)	Placebo Mean (Mean-SEM, Mean+ SEM)	Melatonin Mean (Mean-SEM, Mean+ SEM)	P-values*
1 (1705)	2.3 (1.6, 3.1)	2.4 (1.4, 3.4)	40.2 (23.0, 57.3)	**0.010**
2 (2112)	9.5 (4.9, 14.2)	5.3 (4.0, 6.7)	69.0 (51.1, 86.8)	**0.010**

3 (0732)	17.2 (13.2, 21.3)	16.9 (9.3, 24.4)	197.1143.3, 250.9)	**0.016**

*comparison between melatonin and placebo